Effect of Indigenous Biofertilizer Treatment as Growth Promote on The Vegetative Growth of Tomato

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Abstract: Utilization of beneficial bacteria associated with plants as biofertilizer is proven to increase the vegetative growth of tomato plants and can minimize the use of chemical fertilizers. The treatment of tomato seeds with endophytic bacteria and rhizobacteria conducted at the Agronomy Laboratory of Haluoleo University, Southeast Sulawesi Province. The purpose of this study is to evaluate the ability of micro-organisms to increase tomato plant vegetative growth. This study uses soil media, husk charcoal, and manure in a ratio of 4: 2: 1 (soil: manure: husk charcoal), which is inserted into polybags measuring 30x40 cm and placed in a greenhouse. Three bacterial isolates used are Pseudomonas sp SWRII B02, Bacillus sp CKD061, Pseudomonas sp KeO3, and their combination. The results show that seed treatment with bacteria as a biofertilizer effective in boosting and increasing the vegetative growth of tomato plants. Seed treatment with Bacillus sp CKD061 effectively increases the dry weight and number of plant leaves, while the effective treatment of seeds with Pseudomonas sp SWRII B02 increases the relative growth rate and the root drop ratio. In general, the use of beneficial bacteria as biofertilizer in tomato seeds can increase the number of leaves, stem diameter, dry weight, plant height, relative growth rate, and root ratio of rooted tomato plants is higher than the control group.

Key Word: Biofertilizer, Growth promotes, Vegetative growth, Tomatoes

1 INTRODUCTION

Organic farming activities in supporting sustainable agriculture have been widely applied as an environmentally friendly agricultural model (1). Organic farming is a model approach taken by farmers in producing products that are free of pollutants, carried out continuously, and does not have an impact on environmental damage since it minimizes the use of pesticides and other chemical fertilizers (2). While efforts to improve the health and sustainability of agricultural land management, maximizing organic farming is a priority for sustainable agriculture growth (3). The model of sustainable agricultural land management can be settled through integrated agricultural management by implementing organic agriculture, one of which is by using indigenous bacteria-based fertilizers (4-5). The utilization of organic fertilizers is currently gaining attention as efforts to restore agricultural land productivity continue to decline. Biofertilizers are living microorganisms that are significantly helping plants to provide nutrients for plant growth (6-7). Exploration of potential microorganisms in stimulating plant growth from plant tissues and the Rhizosphere is one of the technologies developed at this time. In addition, the use of biological fertilizer as a biofertilizer in plant growth is expected to act as an organic fertilizer and control pathogens in plants (8). The utilization of non-pathogenic bacteria explored by plants is classified in the Plant Growth Promoting Rhizobacteria (PGPR) group, which is a contribution of biotechnology to increase crop productivity (9-12). Biofertilizer is a community of bacteria inhabiting the Rhizosphere region or in plant tissues that can serve as biocontrols for pathogens and stimulate plant growth to increase crop growth and yield (13). The results of previous studies report that biological fertilizers from the Bacillus spp. and Pseudomonas spp. able to dissolve phosphate (14). In addition, Bacillus sp. It has been reported to be able to synthesize IAA growth hormones (9), gibberellins (15), and cytokinins (9). Meanwhile, Pseudomonas spp can produce IAA (14), gibberellins, and cytokinins (9).

The potential of biological fertilizer as a plant growth booster through its role in dissolving phosphate, nitrogen fixation, and producing growth hormones are expected bacterial characteristics. Therefore, biological fertilizer needs to be evaluated to get biological fertilizer that has potential. This research is aimed at evaluating the bacteria that are active in growing the growth of tomato plants in vegetation. It is anticipated that the successful use of biological fertilizers would increase plant growth and minimize the usage of chemical fertilizers or pesticides.

2 METHODS

This current research was carried out in the Halu Oleo University laboratory and the Halu Oleo University experimental area in the Southeast Province of Sulawesi, Indonesia. It started from August to October 2019. This research activity was designed using a Randomized Block Design (RBD) consisting of 8 treatments with 3 replications, each 8 unit used 3 types of microbes and a combination of A1 = bacterial isolates endophytes Pseudomonas sp SWRII B02, A2 = Rhizosphere bacterial isolates Bacillus sp CKD061, A3 = Rhizosphere bacterial isolates Pseudomonas sp KeO3, A4 = endophytic bacterial isolates Pseudomonas sp SWRII B02 + Rhizosphere bacterial isolates Bacillus sp CKD061, A5 = endophytic bacterial isolates Pseudomonas sp. + Rhizosphere bacterial isolates Pseudomonas sp KeO3, A6 = Rhizosphere bacterial isolates Bacillus sp CKD061 + Rhizosphere bacterial isolates Pseudomonas sp KeO, A7 = endophytic bacterial isolates Pseudomonas sp SWRII B02 + Rhizosphere bacterial isolates Bacillus sp CKD061 + Rhizosphere bacterial isolates Ke Ke, A7 = endophytic bacterial isolates Pseudomonas sp SWRII B02 + Rhizosphere bacterial isolates Bacillus sp CKD061 + Rhizosphere bacterial isolates KeO, A7 = endophytic bacterial isolates Pseudomonas sp SWRII B02 + Rhizosphere bacterial isolates Bacillus sp CKD061 + Rhizosphere bacterial isolates without bacterial treatment. The planting media used consisted of soil, husk charcoal, and manure with a ratio of 4: 2: 1 polybag, size 30x40 cm. The plant media then placed in a

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greenhouse. Observation variables of vegetative growth of tomato plants included leaf number, plant height, stem diameter, dry weight, relative growth rate, and shoot root ratio observed at 7, 14, 21, and 28 days after transplanting (DAT). All collected data were analyzed using variance analysis at a confidence level of 95 percent. If the variance analysis shows a real effect, the Duncan Multiple Range Test (DMRT) will start at the meaning rate of 95 percent.

3 RESULTS

3.1 Dry weight and plant height

Statistical test results of the effect of bacterial isolates on the dry weight of tomato plants observed from day 7 to day 28 after transplanting (DAT) are presented in Table 1. Observation of dry weight on day 7 of DAT did not significantly affect the dry weight of tomato plants, however, on the 14th day of the DAT up to the 28th day of the DAT; bacterial treatment contributed to increasing the dry weight of plants compared to controls. The A2 treatment in the form of Bacillus sp CKD061 Rhizosphere isolates on the 14th day of the DAT and 21st day of the DAT showed an increase in growth every week, and on the 28th day, the plant dry weight was significantly different from the other treatments. The highest dry weight value on the 28th day of DAT was found in the Rhizosphere of Bacillus sp CKD061 with a value of 13,647 gr, which was significantly different from other treatments. Increased plant dry weight is due to a balance of nutrients and hormones in plant tissue. The presence of bacterial isolates can provide optimum and stable nitrogen intake, where it is suspected that bacteria also produce phytohormone, which can increase plant growth. Plant height treatments are presented in Table 2. It appears that from the seven bacterial treatments tested, A4 treatment in the form of endophytic bacterial isolates Pseudomonas sp SWRII B02 + Rhizosphere bacterial isolates Bacillus sp CKD061 can produce higher plants compared to control and other treatments but not significantly different from all treatments. On the 14th day DAT, the bacterial treatment, namely A1, A2, A4, A5, and A6, significantly influenced the height of tomato plants and significantly different from the control but not significantly different between each treatment, similarly on the 21st day DAT which was treated A1, A2, A3, A5 and A6. These results indicate that bacterial inoculants have almost the same effect in increasing tomato plant height.

Table 1. *Effect of bacterial seed treatment on dry weight and tomato plant height*

Treatment	Dry Weight (gr)				Height Plant (cm)			
	7 DAT	14 DAT	21 DAT	28 DAT	7 DAT	14 DAT	21 DAT	28 DAT
A0	0,168a	0,653c	3,010d	6,370d	8,725b	16,808b	33,167b	53,400a
A1	0,195a	0,757c	3,537cd	10,990b	12,125a	22,542a	38,908a	55,183a
A2	0,247a	1,491a	5,260a	13,647a	12,250a	21,650a	38,567a	54,217a
A3	0,222a	1,455a	4,520ab	9,473bc	11,033a	19,917ab	38,458a	56,175a
A4	0,142a	0,760c	3,563cd	9,473bc	12,233a	21,892a	41,100a	59,375a
A5	0,215a	1,190ab	4,243bc	8,273cd	12,942a	22,950a	40,200a	56,783a
A6	0,245a	1,183ab	4,513ab	8,293cd	11,225a	20,792a	37,000ab	55,767a
A7	0,178a	0,993bc	3,747bcd	7,387cd	11,750a	21,242a	39,542a	56,575a

Note: The numbers followed by the same letters in the same column do not indicate any significant difference based on the α = 5% DMRT test; DAT = Day after transplanting.

3.2 Number of Leaves and Stems in Diameter

Treatment of seeds with endophytic and rhizobacterial bacteria and their combinations significantly increased the number of leaves and stems diameters of tomato plants compared with the control group. Among the various bacterial treatment groups studied, A2 treatment in the form of Bacillus sp CKD061 independently administered rhizosphere bacterial isolates were able to increase the amount of tomato plant leaves compared to a control group and was not significantly different from other treatments. Similarly, in the treatment with A4, in the form of endophytic bacterial isolate Pseudomonas sp SWRII B02 + Rhizosphere bacterial isolate Bacillus sp CKD061, the stems of higher tomato plants also increased in diameters, although they were not significantly different from other treatments. On the 28th day DAT, the highest value for the number of leaves was in the A4 treatment with a value of 59.375 strands, and the highest stem diameter was found in the A2 treatment with a value of 54,500 cm. All bacterial treatments had almost the same effect in increasing the number of leaves and stems diameters of tomato plants.

Table 2. *The effect of seed treatment with bacteria on the number of leaves and stems diameter of tomato plants*

Treatment	Number of leaves				Stems Diameter (cm)			
	7 DAT	14 DAT	21 DAT	28 DAT	7 DAT	14 DAT	21 DAT	28 DAT
A0	5,083a	13,583a	29,000b	48,833a	0,357a	0,616b	0,851b	1,075a
A1	6,333a	17,167ab	35,000a	50,917a	0,418a	0,702a	0,933a	1,084a
A2	6,333a	18,583bc	36,167a	54,500a	0,433a	0,670ab	0,932a	1,102a
A3	6,000a	15,167ab	35,333a	51,667a	0,402a	0,685a	0,914a	1,080a
A4	6,333a	17,417ab	31,750ab	53,417a	0,431a	0,707a	0,928a	1,130a
A5	6,333a	17,250ab	34,333a	49,083a	0,418a	0,686a	0,940a	1,078a
A6	6,083a	15,667bc	34,583a	51,250a	0,410a	0,731a	0,977a	1,086a
A7	6,000a	16,667c	33,833a	49,333a	0,409a	0,689a	0,927a	1,073a

Note: The numbers followed by the same letters in the same column do not indicate any significant difference based on the α = 5% DMRT test; DAT = Day after transplanting.

3.3 Relative Growth Rate and Shoot Root Ratio

Endophytic bacterial isolate Pseudomonas sp SWRII B02, which was applied to plant seeds significantly, was also able to increase the relative growth rate and root ratio of rooted tomato plants. The relative growth rate of plants treated with endophytic bacteria Pseudomonas sp SWRII B02 was significantly different than other treatments and controls, but not significantly different from endophytic bacterial isolates of Pseudomonas sp SWRII B02 + isolates of Rhizosphere bacteria Bacillus sp CKD061. Whereas in the variable of shoot toot ratio, endophytic bacteria isolate Pseudomonas sp SWRII B02 and endophytic bacteria isolate Pseudomonas sp SWRII B02 + Rhizosphere bacterial isolates Bacillus sp CKD061 almost resulted in the same effect. The highest relative growth rate value was found in treatment A1 with a value of 0.159 compared with a control group with a value of 0.106. Meanwhile, the highest shoot root ratio in tomato plants was also found in A1 and A4 treatments with values of 10,411 and 10,090, respectively. In general, the shoot root ratio in the bacterial treatment group was better than the control group, and this can be seen in the treatment of A1, A2, A3, A4, and A7 compared with the control group.

Table 3. *The effect of seeds treatment with bacteria on the relative growth rate and shoot root ratio of tomato plants.*

Treatment	Relative Growth Rate			Shoot Root Ratio			
	7 DAT	14 DAT	21 DAT	7 DAT	14 DAT	21 DAT	28 DAT
A0	0,197a	0,198a	0,106bc	7,733	8,226	9,409	8,175
A1	0,195a	0,220a	0,159a	5,644	7,126	9,437	10,411
A2	0,256a	0,181a	0,137abc	6,469	6,208	9,569	9,973
A3	0,273a	0,161a	0,105bc	6,267	5,885	9,406	8,557
A4	0,236a	0,223a	0,141ab	7,244	7,190	7,603	10,090
A5	0,245a	0,182a	0,093bc	6,705	7,959	8,686	8,011
A6	0,221a	0,195a	0,087c	7,048	8,438	7,894	8,084
A7	0,247a	0,191a	0,097bc	7,244	7,600	8,298	9,920

Note: The numbers followed by the same letters in the same column do not indicate any significant difference based on the α = 5% DMRT test; DAT = Day after transplanting.

4 DISCUSSION

This study uses three strains of bacterial isolates, and they are endophytic bacteria Pseudomonas sp SWRII B02, isolates of Bacillus sp CKD061 Rhizosphere bacteria, isolates of Rhudosphere bacteria Pseudomonas sp KeO3 and combinations of those bacteria. Some studies report that the use of bacteria as biological fertilizer can stimulate plant growth (15). Besides, some bacteria also produce IAA and cytokines, increase nitrogen fixation, and can dissolve phosphate (16). In this study, it was found that the application of Pseudomonas sp SWRII B02, Bacillus sp CKD061, Pseudomonas sp KeO3, and their combination had a significant effect in increasing the vegetative growth parameters of tomato plants. The effect of using microbes as biological fertilizers tested on tomato plants can be explained by vegetative growth parameters measurements such as dry weight, plant height, a number of leaves, stems diameter, relative growth rate, and shoot root ratio, which has better growth values than the control group. The application of using bacteria during growth is considered to have a positive role in initiating the vegetative growth of plants. It occurs since bacteria can produce plant hormones such as auxin and cytokinins, which can stimulate plant growth (17-18). It occurs as bacteria can produce plant hormones such as auxin and cytokinins that can stimulate the growth of plants (17-18). The results of this study are also consistent with the study by Zaghloul, which stated that the growth characteristics of plant height, number of leaves, and number of branches in potato tubers increased significantly after inoculation with B. Megaterium var. Phosphaticum 002E (19). The results of vegetative growth as the effect of inoculation with bacteria showed that the Rhizosphere bacterial isolate Bacillus sp CKD061 significantly increased the plant dry weight on day 28 of the DAT with a dry weight value of 13,647 g which was significantly different from the control group, which was 6,370 gr. The contribution of the Bacillus sp CKD061 Rhizosphere bacterial isolate also appeared to increase the number of leaves of tomato plants by 54,500 strands compared with 48.833 strands. Similarly, the endophytic bacterial isolate Pseudomonas sp SWRII B02 + Rhizosphere bacterial isolate Bacillus sp CKD061 caused an increase in plant height of 59.375 cm compared with 53.400 cm control and stem diameter, that was 1,130 cm compared with control, which was 1,075 cm. While endophytic bacteria Pseudomonas sp SWRII B02, it contributed to the increase in the relative growth rate, 0.159 compared to the control group, 0.106, and the shoot root, 10.411 compared with a control group with a value of 8.177. The increase in vegetative growth rate and shoot root ratio were also caused by bacterial inoculation so that the vegetative growth of plants increased and provided more photosynthetic activities and more solute synthesis in plants (20). In general, bacterial treatments gave better results in increasing the vegetative growth of tomato plants compared with the control group. Among all isolates tested, Bacillus sp CKD061 Rhizosphere bacterial isolates and endophytic bacterial isolates Pseudomonas sp SWRII B02 were better at raising vegetative plant growth compared to other treatments and control group, this was because tomato plants' responses to the bacterial treatments used varied. This condition depends on the type and population of bacteria, a combination of bacteria, and environmental conditions (21). Characteristics of the bacteria Bacillus sp CKD061 and Pseudomonas sp SWRII B02 showed a high vegetative growth capacity of plants when used as bio inoculants. Therefore, this isolate can be recommended as a bioinoculant which can be used as a biological fertilizer in the cultivation of crops.

5 CONCLUSION

The treatment of Bacillus sp CKD061 Rhizosphere isolates and endophytic bacterial isolates Pseudomonas sp SWRII B02 were better in promoting vegetative growth of plants than other

treatments and control group. In general, the use of bacteria as biofertilizer in tomato seeds can boost and increase the number of leaves, stem diameter, dry weight, plant height, relative growth rate, and shoot root ratio of tomato plants to be higher than the control group. Further studies are an important step to ensure the achievement of effective bacterial inoculants and can be utilized as growth-promoting agents through field trials. Through field trials, it can be obtained the best ability of bacteria in promoting plant improvement and growth so that it can be applied to the needs of sustainable agricultural cultivation.

6 REFERENCES

1. Mie, A., Andersen, H.R., Gunnarsson, S., Kahl, J., Kesse-Guyot, E., Rembiałkowska, E., Quaglio, G. and Grandjean, P., 2017. Human health implications of organic food and organic agriculture: a comprehensive review. Environmental Health, 16(1), p.111.
2. Souza, R.D., Ambrosini, A., and Passaglia, L.M., 2015. Plant growth-promoting bacteria as inoculants in agricultural soils. Genetics and molecular biology, 38(4), pp.401-419.
3. Yadav, A.N., Verma, P., Singh, B., Chauhan, V.S., Suman, A. and Saxena, A.K., 2017. Plant growth promoting bacteria: biodiversity and multifunctional attributes for sustainable agriculture. Adv Biotechnol Microbiol, 5(5), pp.1-16.
4. Chandra, D., Barh, A. and Sharma, I.P., 2018. Plant growth promoting bacteria: a gateway to sustainable agriculture. In Microbial Biotechnology in Environmental Monitoring and Cleanup (pp. 318-338). IGI Global.
5. Sutariati, G.A.K., Muhidin, Rakian, T.C., Afa, L.O., Widanta, I., Mudi, L., Sadimantara, G.R. and Leomo, S., 2018. The effect of integrated application of pre-plant seed bio-invigoration, organic and inorganic fertilizer on the growth and yield of local upland rice. Bioscience Research, 15(1), pp.160-165.
6. Suman, A., Yadav, A.N. and Verma, P., 2016. Endophytic microbes in crops: diversity and beneficial impact for sustainable agriculture. In Microbial inoculants in sustainable agricultural productivity (pp. 117-143). Springer, New Delhi.
7. Sutariati, G.A.K., Khaeruni, A., Madiki, A., Rakian, T.C., Mudi, L. and Fadillah, N., 2019, May. Seed biopriming with indigenous endophytic bacteria isolated from Wakatobi rocky soil to promote the growth of onion (Allium ascalonicum L.). In IOP Conference Series: Earth and Environmental Science (Vol.260, No. 1, p. 012144). IOP Publishing.
8. Nehra, V. and Choudhary, M., 2015. A review on plant growth promoting rhizobacteria acting as bioinoculants and their biological approach towards the production of sustainable agriculture. Journal of Applied and Natural Science, 7(1), pp.540-556.
9. Noumavo, P.A., Agbodjato, N.A., Baba-Moussa, F., Adjanohoun, A. and Baba-Moussa, L., 2016. Plant growth promoting rhizobacteria: Beneficial effects for healthy and sustainable agriculture. African Journal of Biotechnology, 15(27), pp.1452-1463.
10. Kade, S.G.A. and Jusoff, K., 2013. Effectiveness of bioinvigoration technologies on seed viability and vigor of cocoa (Theobroma cacao L.). World Applied Sciences Journal, 26, pp.31-36.
11. Sutariati, G.A.K., Bande, L.O.S., Khaeruni, A., Mudi, L. and Savitri, R.M., 2018, February. The effectiveness of preplant seed bio-invigoration techniques using Bacillus sp. CKD061 to improving seed viability and vigor of several local upland rice cultivars of Southeast Sulawesi. In IOP Conference Series: Earth and Environmental Science (Vol. 122, No. 1, p. 012031). IOP Publishing.
12. Sutariati, G.A.K., Arif, N. and Rakian, T.C., 2017. Persistency and Seed Breaking Dormancy on Local Upland Rice of Southeast Sulawesi, Indonesia. Pakistan journal of biological sciences: PJBS, 20(11), pp.563-570.
13. Yadav, A.N., Kumar, R., Kumar, S., Kumar, V., Sugitha, T., Singh, B., Chauhan, V.S., Dhaliwal, H.S. and Saxena, A.K., 2017. Beneficial microbiomes: biodiversity and potential biotechnological applications for sustainable agriculture and human health. J Appl Biol Biotechnol, 5(6), pp.45-57.
14. Ilyas, S., Asie, K.V. and Sutariati, G.A.K., 2014, August. Biomatriconditioning or biopriming with biofungicides or biological agents applied on hot pepper (Capsicum annuum L.) seeds reduced seedborne Colletotrichum capsici and increased seed quality and yield. In XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): 1105 (pp. 89-96).
15. Kumar, M., Tomar, R. S., Lade, H., & Paul, D. (2016). ethylotrophic bacteria in sustainable agriculture. World Journal of Microbiology and Biotechnology, 32(7), 120.
16. Kuan, K.B., Othman, R., Rahim, K.A. and Shamsuddin, Z.H., 2016. Plant growth-promoting rhizobacteria inoculation to enhance vegetative growth, nitrogen fixation and nitrogen remobilisation of maize under greenhouse conditions. PloS one, 11(3), p.e0152478.
17. Gouda, S., Kerry, R.G., Das, G., Paramithiotis, S., Shin, H.S. and Patra, J.K., 2018. Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. Microbiological research, 206, pp.131-140.
18. La Fua, J., Sabaruddin L., Bande, L.S., Leomo, S., 2019. The Potential of Indigenous Endophyte Bacteria to Promote Local Tomato Growth Isolated From Dry Land in Muna, Southeast Sulawesi, Indonesia. International Journal Of Scientific & Technology Research, Volume 8, Issue 12, December 2019.
19. Zaghloul, R.A., 2002, October. Biofertilization and organic manuring efficiency on growth and yield of potato plants (Solanum tuberosum L.). In Recent Technologies in Agriculture. Proceedings of the 2nd Congress. Fac. Agric., Cairo Univ (pp. 28-30).
20. Dawwam, G.E., Elbeltagy, A., Emara, H.M., Abbas, I.H. and Hassan, M.M., 2013. Beneficial effect of plant growth promoting bacteria isolated from the roots of potato plant. Annals of Agricultural Sciences, 58(2), pp.195-201.
21. Hayat, R., Ali, S., Amara, U., Khalid, R. and Ahmed, I., 2010. Soil beneficial bacteria and their role in plant growth promotion: a review. Annals of microbiology,60(4), pp.579-598.